Storage and Bacterial Contamination Effects on Myofibrillar Proteins and Shear Force of Beef

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ABSTRACT

Thirty-two steaks from the longissimus muscle, fifth rib to third lumbar vertebra, were obtained from youthful carcass beef. Half were sterilized by ultraviolet light and all vacuum packaged and stored for 1, 14, 28 or 57 days at 2°C. After storage, steaks were examined for microbial populations, myofibril fragmentation index (MFI), cooking characteristics and shear force (SF). Aerobic and anaerobic counts decreased during storage. Psychrotrophic counts were low throughout. Sterilization had no effect on SF or MFI. Cooking loss tended (P < 0.09) to increase with time of storage. SF values decreased and MFI values increased through day 14, but remained relatively constant after that. Results of SDS-PAGE, SF and MFI indicate major changes in proteolysis of myofibrils and tenderness were completed by day 14.

INTRODUCTION

RECENT RESULTS indicate most postmortem proteolysis occurs within 72 hr postmortem, and associated improvement in tenderness has occurred by that time (Koohmaraie et al. 1987). Whipple et al. (1990) observed that after 14 days aging, meat from *Bos indicus* crossbred cattle was less tender than meat from *Bos taurus* cattle, and no significant improvement occurred after 72 hr storage at 2°C. Research results by Koohmaraie and coworkers (Koohmaraie et al. 1988a; Koohmaraie, 1990) demonstrated that improved tenderness associated with aging was the result of proteolysis of muscle proteins. Whipple et al. (1990) and Shackelford et al. (1990) observed that Calcium-Dependent Protease (CDP) inhibitor measured after 24 hr storage at 0°C was the biological trait most highly associated with tenderness after 14 days storage at 2°C. They speculated that CDP inhibitor may be an important regulator of tenderness.

Based on results of those experiments, it was hypothesized that most changes in tenderness of refrigerated meat resulting from endogenous factors (e.g., proteases) occurred by 14 days postmortem storage. Beyond this, additional changes in muscle fiber or connective tissue integrity probably occur from exogenous sources (e.g., microbiological). Therefore, our objectives were to determine changes that occur to muscle fiber integrity while stored under sterile and non-sterile conditions and at refrigerated temperatures during 57-days storage.

MATERIAL & METHODS

THIRTY-TWO 2.5-cm steaks were obtained 24 h postmortem from the longissimus muscle between the fifth rib and third lumbar vertebra from two Hereford by Angus crossbred heifers. They were fed alfalfa haylage, corn silage diet for 4 mo after weaning, and then fed a corn, corn silage diet until slaughter at age 15 mo. Heifers averaged 452 kg live weight at slaughter and yielded carcasses with 1.65 cm adjusted fat thickness, 77 cm² longissimus muscle area and 286 kg carcass weight. Carcasses were A maturity and graded average Choice or high Select (USDA, 1989). No dark-coarse banding was observed. Steaks were stratified by anatomical location, and equal numbers from each location were assigned to treatments.

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Preparation of sterile steaks

Half the steaks were sterilized on sterile aluminum foil on the work surface of a laminar flow hood. Shortwave ultraviolet sterilizing lamps (254 nm) in the hood were turned on, and steaks were exposed at 60 cm and ambient temperature 45 min on each side. After irradiation, edges of the steaks were flamed about 5 sec with a Bunsen burner to destroy bacteria which may not have been exposed to direct ultraviolet irradiation.

Within sterilized and nonsterilized groups, steaks were assigned to one of four storage treatments of 1, 14, 28 or 57 days. Steaks were individually wrapped in oxygen impermeable polyethylene (3 mil thickness) bags, sealed under vacuum and stored at 2°C. Steaks were not exposed to light during storage. After storage, bacterial contamination, myofibril fragmentation index (MFI), cooking loss, cooking time and shear force (SF) of 1.3 cm cooked meat cores were determined. The MFIs were determined on uncooked longissimus muscle samples according to Culler et al. (1978).

Bacteriological analysis

A 2.0 × 2.0-cm section was aseptically excised from each steak surface using a sterile scalpel and forceps to an approximate depth of 3 mm. The samples were homogenized in sterile Butterfield's phosphate buffer (Pertel and Kazanas, 1984) for 2 min in a Stomacher 400 (Tekmar, Inc., Cincinnati, OH), then serially diluted in phosphate buffer. Total aerobic and psychrotrophic bacteria counts were determined using Tryptic Soy Agar (TSA, Difco, Detroit, MI) and the pour-plate technique (Busta et al., 1984), incubating the plates at 32°C for 48 hr and 5°C for 7 days, respectively. Proteolytic bacteria were enumerated using TSA with 10% (wt/vol) nonfat dry milk and the surface plate technique (Busta et al., 1984), incubating the plates at 32°C for 48 hr. Anaerobic bacteria were enumerated using Brain Heart Infusion Agar (BHI, Difco) and the pour-plate technique. BHI plates were incubated in a GasPak Plus anaerobe system (BBL Microbiology Systems, Cockeysville, MD) at 32°C for 48 hr.

Sodium-dodecylsulfate-polyacrylamide gel electrophoresis (SDS-PAGE)

The effect of treatment on the proteolysis of myofibrillar proteins during a 57-d storage at 2°C was examined by SDS-PAGE. Myofibrils were isolated from longissimus muscle after 1, 14, 28 and 57 days storage according to the procedure described by Goll et al. (1974). Protein concentrations were determined (Gornall et al., 1949) and 60 μg of myofibrillar proteins were electrophoresed on 7.5 to 15% discontinuous slab gel (Hames and Rickwood, 1982). The ratio of acrylamide to bisacrylamide was 75:1. The acrylamide solution (30%) contained 50% glycerol.

Cooking and shear force

Steaks were broiled to internal temperature 40°C, turned over, and broiled to internal temperature 70°C on "Open-Hearth" electric broilers (Model 450, Farberware, Bronx, NY) for determination of SF. Internal temperatures were monitored by an iron-constantan thermocouple probe attached to a Honeywell potentiometer (Model 112). Weights were recorded before and after cooking for determination of cooking loss (%). Also, cooking time (min) was observed. Steaks were tempered for 24 hr at 4°C and six 1.3-cm cores were removed from each steak parallel to longitudinal orientation of the muscle fibers. Cores were sheared with a Warner-Bratzler shear device attached to an Instron 1132/Microcon II Universal Testing Instrument (Instron Corp., Canton, MA). The crosshead speed was 5 cm/min and the fail criterion was 75%.

Table 1—Means and SE of textural and microbiological traits of meat for sterilization and storage treatments

| Treatment	N	MFI	Shear force kg	Cooking loss %	Cooking time min	Aerobic count CFU/cm²ᵃ	Proteolytic CFU/cm²	Psychrotrophic CFU/cm²	Anaerobic CFU/cm²
Time (hr)									
1	8	52.8	5.34	22.3	27.0	1.62	0.50	0.47	1.50
14	8	67.6	4.01	23.7	30.4	1.99	0.31	<0.25	0.44
28	8	73.9	3.64	26.1	26.3	2.06	<0.25	<0.25	<0.25
57	8	80.8	3.61	27.6	33.1	<0.25	<0.25	0.44	<0.25
SE		3.4	0.24	2.1	3.2	0.14	0.23	0.45	0.34
Least significant difference		7.0[b]	0.49[b]			0.29[b]			0.69[b]
Nonsterile	16	68.3	4.27	25.6	29.2	2.56[c]	0.41	0.45	0.97[c]
Sterile	16	69.2	4.02	24.2	29.2	0.28[d]	<0.25	<0.25	<0.25[d]
SE		1.7	0.12	1.0	1.6	0.07	0.11	0.23	0.17

[a] Log_{10} colony forming units/cm².
[b] Means among time periods differ ($P < 0.01$).
[c,d] Means without common superscripts differ ($P < 0.01$).
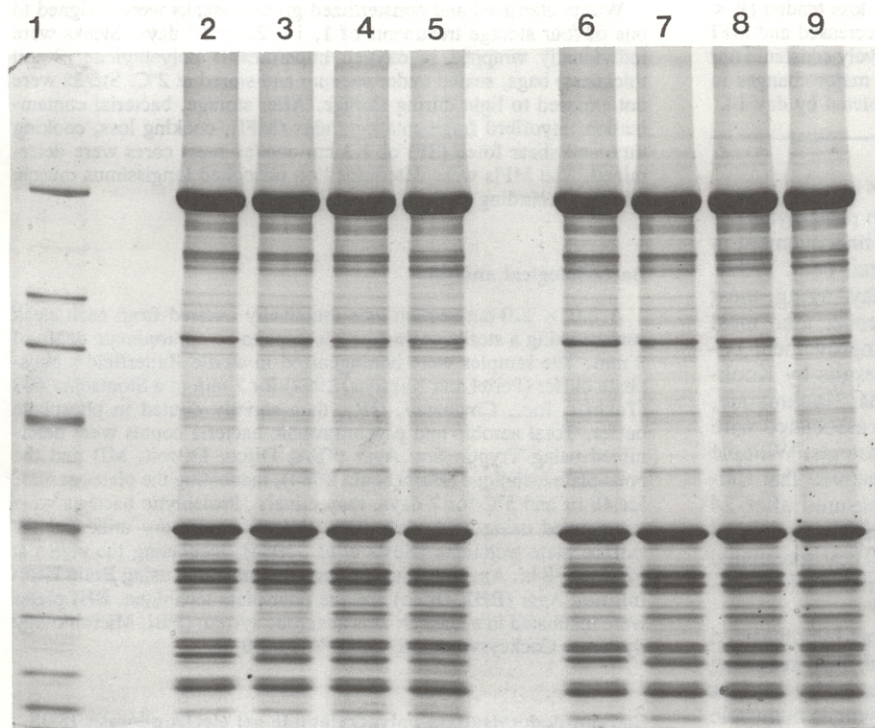
Fig. 1—SDS-PAGE of myofibrils isolated from longissimus muscles on sterile (lanes 2, 3, 4, and 5) and nonsterile steaks (lanes 6, 7, 8, and 9) after 1, 14, 28, and 57 days of storage at 2°C. Lane 1 contains molecular-weight standards (Bio-Rad) corresponding to 200 kd (myosin), 116.2 kd (E. coli beta-galactosidase), 97.4 kd (rabbit muscle phosphorylase b), 66.2 kd (bovine serum albumin), 42.7 kd (hen egg white ovalbumin), 31 kd (bovine carbonic anhydrase), 21.5 kd (soybean trypsin inhibitor) and 14.4 kd (hen egg white lysozyme) from top to bottom, respectively. Arrows A, B and C correspond to the position of 55 kd polypeptide (probably desmin), troponin-T and 28 to 32 kd polypeptides, respectively. Samples (60 µg of purified myofibrillar proteins) were electrophoresed on 7.5 to 15% gradient slab gels and stained with coomassie blue R-250.

Analysis of data

Data were analyzed by least-squares procedures (SAS, 1985). A split-plot model was used with animal and sterilization as whole plot effects, time of storage as subplot effects and two- and three-way interactions. Least-significant differences (LSD) were computed for comparisons among subclass means. All tests were conducted at the 99% level of confidence.

RESULTS & DISCUSSION

Microbiology

A significant sterilization by time of storage interaction was observed for total plate count (TPC) and anaerobic organisms (data not shown). Aerobic counts decreased in the nonsterile group over storage (periods: $1 = 3.24$, $14 = 3.99$, $28 = 3.00$ and 57 days $= <0.25$ log_{10}). In the 28-day group of the sterile treatment, 1.12 log_{10} aerobic counts were observed, but no organisms were detected with aerobic count for the sterile treatments at days 1, 14 or 57. Detectable aerobic counts were obtained for two of the four "sterile" steaks analyzed at day 28, resulting in a mean aerobic count of 1.12 log_{10}. This may have been attributable to improper sterilization, recovery of radiation-injured cells, or contamination during sampling.

Similarly, the number of anaerobic count in the nonsterile treatment decreased from 3.00 to <0.25 log_{10} over the trial. No anaerobic organisms were observed in the sterilized meat (Table 1). Other than the possibility of effects of storage at 2°C, the reason for the decrease in aerobic and anaerobic counts over the 57-day period was not clear. Although psychrotrophic organisms persisted throughout the trial, the count was very low and did not differ from the sterile group (Table 1). Initial aerobic counts on the steaks were relatively low and consisted almost exclusively of mesophilic species. This was indicated by the very low psychrotrophic counts which were detected infrequently on the non-sterile steaks, with many individual steaks having no detectable psychrotrophs. Anaerobic bacteria were detected only on days 1 and 14 with the non-sterile group, so that at 28 days, the microflora consisted exclusively of mesophilic, aerobic bacteria. The pH of the tissue, although not determined in these experiments, would probably be in the pH 5.5 to 5.8 range (Koohmaraie et al., 1988b). The combination of sub-optimal pH, vacuum packaging and low tem-

perature were probably the reason for decline in bacterial numbers from 28 to 57 days.

Cooking traits

No interactions were observed for cooking losses or cooking times. Also, no differences were observed between sterilized and non-sterilized meat samples for cooking characteristics (Table 1). A trend (P < 0.09) was observed toward increased percent cooking loss associated with storage time. The results indicate possible increased cooking losses associated with length of time of storage.

Textures

No interactions nor sterilization treatment effects were observed for MFI or SF values (Table 1), which declined (P < 0.01) through day 14, tended to decrease after that, but were constant after day 28. These results indicated that storage of meat after 14 d would not appreciably improve tenderness. Marsh et al. (1981) and Koohmaraie et al. (1987) observed that the greatest improvement in tenderness occurred during the first 1 to 3 days of storage and very little change was evident after 5 days.

As with SF, MFI values increased through day 14 of the trial with only a nonsignificant subsequent increase in the traits beyond 14 d (Table 1). Results indicated that more than 50% of proteolysis was complete by 14 d storage. The MFI is indicative of myofibril proteolysis and has been correlated with tenderness (Parrish, 1977).

The MFI and gel patterns explained the decrease in rate of improvement in SF values over time. When treatment effects on postmortem proteolysis of myofibrillar proteins were determined (Fig. 1), results indicated that between 1 and 14 days significant changes occurred in those from nonsterile steaks. Changes included the disappearance of a polypeptide with molecular weight 55 kd (probably desmin) and troponin-T, and the appearance of a group of polypeptides with molecular weights 28 - 32 kd. Results were in agreement with those reported previously (Goll et al. 1983; Koohmaraie, 1988). However, those changes occurred in sterile steaks between 14 and 28 days storage. No significant changes occurred in myofibrillar proteins beyond 28 days storage. Because microbial contamination is a surface phenomenon and samples were obtained from the interior, it is doubtful that such differences may be due to microbial population. Results of SDS-PAGE, MFI and SF, collectively, indicated that the majority of the postmortem changes leading to improvement in tenderness had occurred by day 14, and that any minor changes occurred beyond this date.

CONCLUSIONS

MEAT under sanitary conditions and refrigeration was stored 57 d without increases in microbial contamination. Microbial populations had no effect on myofibril proteolysis or SF values. Major changes in SF values were made by 14 d storage and only minor appreciable changes occurred after the first 14 d storage.

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